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(71) Applicant: OREGON HEALTH SCIENCES UNIVERSITY [US/US]; 3181 S.W. Sam Jackson Park Road, Portland, OR 97201 (US).

(71)(72) Applicants and Inventors: YATVIN, Milton, B. [US/US]; 5226 S.W. Northwood Avenue, Portland, OR 97201 (US). STOWELL, Michael, H., B. [/US]; Catalina 438 #105, Pasadena, CA 91106 (US). MEREDITH, Michael, J. [US/US]; 3 S.W. Offenbach Place, Lake Oswego, OR 97035 (US).

(74) Agent: NOONAN, Kevin, E.; McDonnell Boehnen Hulbert & Berghoff, 300 South Wacker Drive, Chicago, IL 60606 (US).

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(57) Abstract

This invention herein describes a method of facilitating the entry of drugs into cells and tissues at physiologically protected sites at pharmicokinetically useful levels and also a method of targeting drugs to specific organelles within the cell. This polar lipid/drug conjugate targeting invention embodies an advance over other drug targeting methods known in the prior art, because the invention provides drug concentrations in such physiologically protected sites that can reach therapeutically-effective levels after administration of systemic levels much lower than are currently administered to achieve a therapeutic dose. This technology is appropriate for use with psychotropic, neurotropic and neurological drugs, agents and compounds, for rapid and efficient introduction of such agents across the blood-brain barrier. Further, the invention provides means for retention and prolonged enzymatic release of psychotropic, neurotropic and neurological drugs, agents and compounds comprising the conjugates of the invention, in the brain and central nervous system.

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COVALENT POLAR LIPID CONJUGATES FOR TARGETING

BACKGROUND OF THE INVENTION

1. Field of the Invention

A major goal in the pharmacological arts has been the development of methods and compositions to facilitate the specific delivery of therapeutic and other agents to the appropriate cells and tissues that would benefit from such treatment, and the avoidance of the general physiological effects of the inappropriate delivery of such agents to other cells or tissues of the body. One common example of the need for such specificity is in the field of neurologic agent therapy for the treatment of diseases of the central nervous system, particularly the brain, which is protected by a layer of endothelial cells and other structures collectively known as the blood-brain barrier. In the pharmacological and neurologic arts, it is well-recognized that the inability to deliver effective amounts of neurotropic, psychotropic and anticonvulsant drugs and agents across the blood-brain barrier severely limits the therapeutic efficacy of such pharmaceutical compounds and can prevent treatment of neurologic disease. In addition, the use of even effective neurologic agents is further limited by systemic toxicity resulting from the high systemic concentrations that must be administered to achieve a therapeutic concentration of such agents in the brain, central nervous system and other neurological structures. Similar considerations apply in other organs and tissues in mammals that are protected by such blood-related barriers, such as the testes.

In addition, it is recognized in the medical arts that certain subcellular organelles are the sites of pharmacological action of certain drugs or are involved in the biological response to certain stimuli. Specific delivery of diagnostic or therapeutic compounds to such intracellular organelles is thus desirable to increase the specificity and effectiveness of such clinical diagnostic or therapeutic techniques.

Drug Targeting

It is desirable to increase the efficiency and specificity of administration of a therapeutic agent to the cells of the relevant tissues protected by physiological barriers (*i.e.*, such as the blood-brain barrier) in a variety of pathological states. This is particularly important as relates to psychotropic, neurological and neurotropic agents.

Such agents typically have systemic effects, including renal and hepatotoxicity, hematopoietic suppression, teratogenic capacity, partitioning into breast milk and other pleiotropic cytotoxic effects that damage or otherwise deleteriously impact on uninvolved cells and tissues. This is particularly the case in delivering psychotropic, neurotropic and neurological agents to physiologically protected sites, since high systemic concentrations of such agents are required to promote partitioning of a sufficient amount of the psychotropic, neurotropic and neurological agents into the protected sites to achieve a therapeutic result. Thus, an efficient delivery system which would enable the delivery of such drugs specifically to cells and tissues in such physiologically protected sites would increase the efficacy of treatment and reduce the associated "side effects" of such drug treatments, and also serve to reduce morbidity and mortality associated with clinical administration of such drugs.

Numerous methods for enhancing the biological activity and the specificity of drug action have been proposed or attempted (see, for example, Kreeger, 1996, *The Scientist*, September 16, 1996, p. 6). To date, however, efficient or specific drug delivery remains to be predictably achieved.

U.S. Patent No. 5,017,566, issued May 21, 1991 to Bodor disclose β- and γ-cyclodextrin derivatives comprising inclusion complexes of lipoidal forms of dihydropyridine redox targeting moieties.

U.S. Patent No. 5,023,252, issued June 11, 1991 to Hseih disclose the use of pharmaceutical compositions comprising a neurologically active drug and a compound for facilitating transport of said drug across the blood-brain barrier including a macrocyclic ester, diester, amide, diamide, amidine, diamidine, thioester, dithioester, thioamide, ketone or lactone.

U.S. Patent No. 5,024,998, issued June 18, 1991 to Bodor disclose parenteral solutions of aqueous-insoluble drugs with β- and γ-cyclodextrin derivatives.

U.S. Patent No. 5,039,794, issued August 13, 1991 to Wier *et al.* disclose the use of a metastatic tumor-derived egress factor for facilitating the transport of compounds across the blood-brain barrier.

U.S. Patent No. 5,112,863, issued May 12, 1992 to Hashimoto *et al.* disclose the use of N-acyl amino acid derivatives as antipsychotic drugs for delivery across the blood-brain barrier.

U.S. Patent No. 5,124,146, issued June 23, 1992 to Neuwelt disclose a method

for delivery of therapeutic agents across the blood-brain barrier at sites of increase permeability associated with brain lesions.

U.S. Patent No. 5,153,179, issued October 6, 1992 to Eibl disclose acylated glycerol and derivatives for use in a medicament for improved penetration of cell membranes.

U.S. Patent No. 5,177,064, issued January 5, 1993 to Bodor disclose the use of lipoidal phosphonate derivatives of nucleoside antiviral agents for delivery across the blood-brain barrier.

U.S. Patent No. 5,254,342, issued October 19, 1993 to Shen *et al.* disclose receptor-mediated transcytosis of the blood-brain barrier using the transferrin receptor in combination with pharmaceutical compounds that enhance or accelerate this process.

U.S. Patent No. 5,258,402, issued November 2, 1993 to Maryanoff disclose treatment of epilepsy with imidate derivatives of anticonvulsive sulfamates.

U.S. Patent No. 5,270,312, issued December 14, 1993 to Glase *et al.* disclose substituted piperazines as central nervous system agents.

U.S. Patent No. 5,284,876, issued February 8, 1994 to Shashoua *et al.*, disclose fatty acid conjugates of dopanergic drugs for tardive dyskinesia.

U.S. Patent No. 5,389,623, issued February 14, 1995 to Bodor disclose the use of lipoidal dihydropyridine derivatives of anti-inflammatory steroids or steroid sex hormones for delivery across the blood-brain barrier.

U.S. Patent No. 5,405,834, issued April 11, 1995 to Bundgaard *et al.* disclose prodrug derivatives of thyrotropin releasing hormone.

U.S. Patent No. 5,413,996, issued May 9, 1995 to Bodor disclose acyloxyalkyl phosphonate conjugates of neurologically-active drugs for anionic sequestration of such drugs in brain tissue.

U.S. Patent No. 5,434,137, issued July 18, 1995 to Black disclose methods for the selective opening of abnormal brain tissue capillaries using bradykinin infused into the carotid artery.

U.S. Patent No. 5,442,043, issued August 15, 1995 to Fukuta *et al.* disclose a peptide conjugate between a peptide having a biological activity and incapable of crossing the blood-brain barrier and a peptide which exhibits no biological activity and is capable of passing the blood-brain barrier by receptor-mediated endocytosis.

U.S. Patent No. 5,466,683, issued November 14, 1995 to Sterling *et al.* disclose

water soluble analogues of the anticonvulsant Tegretol® (carbamazepine) for the treatment of epilepsy.

U.S. Patent No. 5,525,727, issued June 11, 1996 to Bodor disclose compositions for differential uptake and retention in brain tissue comprising a conjugate of a narcotic analgesic and agonists and antagonists thereof with a lipoidal form of dihydropyridine that forms a redox salt upon uptake across the blood-brain barrier that prevents partitioning back to the systemic circulation thereafter.

International Patent Application Publication Number WO85/02342, published 6 June 1985 for Max-Planck Institute disclose a drug composition comprising a glycerolipid or derivative thereof.

International Patent Application Publication Number WO89/11299, published 30 November 1989 for State of Oregon disclose a chemical conjugate of an antibody with a an enzyme which is delivered specifically to a brain lesion site for activating a separately-administered neurologically-active prodrug.

International Patent Application Publication Number WO91/04014, published 4 April 1991 for Synergen, Inc. disclose methods for delivering therapeutic and diagnostic agents across the blood-brain barrier by encapsulating said drugs in liposomes targeted to brain tissue using transport-specific receptor ligands or antibodies.

International Patent Application Publication Number WO91/04745, published 18 April 1991 for Athena Neurosciences, Inc. disclose transport across the blood-brain barrier using cell adhesion molecules and fragments thereof to increase the permeability of tight junctions in vascular endothelium.

International Patent Application Publication Number WO91/14438, published 3 October 1991 for Columbia University disclose the use of a modified, chimeric monoclonal antibody for facilitating transport of substances across the blood-brain barrier.

International Patent Application Publication Number WO94/01131, published 20 January 1994 for Eukarion, Inc. disclose lipidized proteins, including antibodies.

International Patent Application Publication Number WO94/03424, published 17 February 1994 for Ishikura *et al.* disclose the use of amino acid derivatives as drug conjugates for facilitating transport across the blood-brain barrier.

International Patent Application Publication Number WO94/06450, published 31 March 1994 for the University of Florida disclose conjugates of neurologically-active

drugs with a dihydropyridine-type redox targeting moiety and comprising an amino acid linkage and an aliphatic residue.

International Patent Application Publication Number WO94/02178, published 3 February 1994 for the U.S. Government, Department of Health and Human Services disclose antibody-targeted liposomes for delivery across the blood-brain barrier.

International Patent Application Publication Number WO95/07092, published 16 March 1995 for the University of Medicine and Dentistry of New Jersey disclose the use of drug-growth factor conjugates for delivering drugs across the blood-brain barrier.

International Patent Application Publication Number WO96/00537, published 11 January 1996 for Southern Research Institute disclose polymeric microspheres as injectable drug-delivery vehicles for delivering bioactive agents to sites within the central nervous system.

International Patent Application Publication Number WO96/04001, published 15 February 1996 for Molecular/Structural Biotechnologies, Inc. disclose omega-3-fatty acid conjugates of neurologically-active drugs for brain tissue delivery.

International Patent Application Publication Number WO96/22303, published 25 July 1996 for the Commonwealth Scientific and Industrial Research Organization disclose fatty acid and glycerolipid conjugates of neurologically-active drugs for brain tissue delivery.

An additional challenge in designing an appropriate drug delivery scheme is to include within the drug conjugate a functionality which could either accelerate or reduce the rate at which the drug is released upon arrival at the desired site. Such a functionality would be especially valuable if it allowed differential rates of drug release, or specific release only at the appropriate drug target site.

There remains a need in the art for an effective means for the specific delivery of biologically-active compounds, particularly psychotropic, neurotropic and neurological drugs and agents, to physiologically restricted or protected sites. Advantageous embodiments of such delivery means are formulated to efficiently deliver the biologically-active compound to a physiologically-protected site, such as the brain or central nervous system, while minimizing hepatic and renal uptake of the agent or hematopoietic insult resulting therefrom.

SUMMARY OF THE INVENTION

The present invention is directed to an improved method for delivering biologically-active compounds, particularly drugs including preferably psychotropic, neurotropic and neurologically-acting drugs, to physiologically protected sites in an animal *in vivo*. This delivery system achieves specific delivery of such biologically-active compounds through conjugating the compounds with a polar lipid carrier. This invention has the specific advantage of facilitating the entry of such compounds into cells and tissues protected by such physiological barriers as the blood-brain barrier *via* a polar lipid carrier, achieving effective intracellular concentration of such compounds more efficiently and with more specificity than conventional delivery systems.

The invention provides compositions of matter comprising a biologically-active compound covalently linked to a polar lipid carrier molecule. Preferred embodiments also comprise a spacer molecule having two linker functional groups, wherein the spacer has a first end and a second end and wherein the lipid is attached to the first end of the spacer through a first linker functional group and the biologically-active compound is attached to the second end of the spacer through a second linker functional group. In preferred embodiments, the biologically-active compound is a drug, most preferably a psychotropic, neurotropic or neurologically-acting drug or agent, or an antioxidant. Preferred polar lipids include but are not limited to acyl- and acylated carnitine, sphingosine, ceramide, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, cardiolipin and phosphatidic acid. Preferred biologically-active compounds include neurotropic agents such as L-dopa, hydroxytryptamine and metabolites thereof; amantadine, benztropine, bromocryptine, diphenhydramine, levadopa (a particularly preferred embodiment) and combinations thereof (*e.g.*, with carbidopa as provided as Sinemet®); pergolid, trihexphenidyl, ethosuximide, valproic acid, carbamazepine (*e.g.*, Tegretol®) and, in a particularly preferred embodiment, the 10- or 11-hydroxy analogues of carbamazepine; primidone, gabapentin in a particularly preferred embodiment; lamotrigine in a particularly preferred embodiment; felbamate, paramethadione and trimethadione; phenothiazines, thioxanthemes and related compounds; clozapine, haldoperidol, loxapine (Loxitane®), benzodiazapene antidepressants of the norepinephrine reuptake inhibitor type; monoamine oxidase inhibitors; anticonvulsants and antiepileptic agents, such as phenytoin (*e.g.*, Dilantin, Dilabid, or Zentropil); and antioxidants such as

carotenes, glutathione and N-acetylcysteine. Pharmaceutical compositions comprising the drug/polar lipid conjugates of the invention are also provided.

The invention also provides compositions of matter comprising a biologically-active compound covalently linked to a lipid, most preferably a polar lipid, carrier molecule *via* a spacer molecule wherein the spacer allows the biologically-active compound to act without being released at an intracellular site. In these embodiments of the invention, the first linker functional group attached to the first end of the spacer is characterized as "strong" and the second linker functional group attached to the second end of the spacer is characterized as "weak", with reference to the propensity of the covalent bonds between each end of the spacer molecule to be broken.

In other embodiments of the compositions of matter of the invention, the spacer allows the facilitated hydrolytic release of the biologically-active compound at an intracellular site. Other embodiments of the spacer facilitate the enzymatic release of the biologically-active compound at an intracellular site. In particularly preferred embodiments, the spacer functional group is hydrolyzed by an enzymatic activity found in brain tissue, including neuronal, glial and other brain cell types, preferably an esterase and most preferably an esterase having a differential expression and activity profile in the appropriate target cell type. In additional preferred embodiments, specific release of biologically-active compounds is achieved by enzymatic or chemical release of the biologically-active compound by extracellular cleavage of a cleavable linker moiety *via* an enzymatic activity specific for brain tissue, with resulting specific uptake of the released psychotropic, neurotropic or neurological agent by the appropriate cell in said tissue.

In another embodiment of this aspect of the invention, the spacer molecule is a peptide of formula $(\text{amino acid})_n$, wherein n is an integer between 2 and 25, preferably wherein the peptide comprises a polymer of one or more amino acids.

In other embodiments of the compositions of matter of the invention, the biologically-active compound of the invention has a first functional linker group, and a lipid, most preferably a polar lipid, carrier has a second functional linker group, and the compound is covalently linked directly to the lipid carrier by a chemical bond between the first and second functional linker groups. In preferred embodiments, each of the first and second functional linker groups is a hydroxyl group, a primary or secondary amino group, a phosphate group or substituted derivatives thereof or a

carboxylic acid group. In preferred embodiments, the biologically active compound is a drug, most preferably an a psychotropic, neurotropic or neurological drug or agent. Preferred polar lipids include but are not limited to acyl- and acylated carnitine, sphingosine, ceramide, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, cardiolipin and phosphatidic acid.

In another aspect of the invention is provided compositions of matter comprising a drug, most preferably an a psychotropic, neurotropic or neurological drug or agent, covalently linked to a polar lipid carrier molecule. Preferred embodiments also comprise a spacer molecule having two linker functional groups, wherein the spacer has a first end and a second end and wherein the lipid is attached to the first end of the spacer through a first linker functional group and the drug is attached to the second end of the spacer through a second linker functional group. Preferred embodiments of the invention are provided wherein the drug is a psychotropic, neurotropic or neurological drug or agent. Preferred polar lipids include but are not limited to acyl- and acylated carnitine, sphingosine, ceramide, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, cardiolipin and phosphatidic acid. Preferred psychotropic, neurotropic or neurological drugs or agents comprising the conjugates of the invention include L-dopa, hydroxytryptamine and metabolites thereof; amantadine, benztropine, bromocryptine, diphenhydramine, levadopa (a particularly preferred embodiment) and combinations thereof (*e.g.*, with carbidopa as provided as Sinemet®); pergolid, trihexphenidyl, ethosuximide, valproic acid, carbamazepine (*e.g.*, Tegretol®) and, in a particularly preferred embodiment, the 10- or 11-hydroxy analogues of carbamazepine; primidone, gabapentin in a particularly preferred embodiment; lamotrigine in a particularly preferred embodiment; felbamate, paramethadione and trimethadione; phenothiazines, thioxanthenes and related compounds; clozapine, haldoperidol, loxapine (Loxitane®), benzodiazapene antidepressants of the norepinephrine reuptake inhibitor type; monoamine oxidase inhibitors; anticonvulsants and antiepileptic agents, such as phenytoin (*e.g.*, Dilantin, Dilabid, or Zentropil); and antioxidants such as carotenes, glutathione and N-acetylcysteine. Pharmaceutical compositions comprising the drug/polar lipid conjugates of the invention are also provided.

The invention also provides compositions of matter comprising a psychotropic,

neurotropic or neurological drug or agent, covalently linked to a polar lipid carrier molecule *via* a spacer molecule, wherein the spacer allows the drug to act without being released at an intracellular site. In these embodiments of the invention, the first linker functional group attached to the first end of the spacer is characterized as "strong" and the second linker functional group attached to the second end of the spacer is characterized as "weak", with reference to the propensity of the covalent bonds between each end of the spacer molecule to be broken.

In other embodiments of the compositions of matter of the invention, the spacer allows the facilitated hydrolytic release of a psychotropic, neurotropic or neurological drug or agent at an intracellular site. Other embodiments of the spacer facilitate the enzymatic release of the psychotropic, neurotropic or neurological drug or agent of the invention at an intracellular site. In particularly preferred embodiments, the spacer functional group is hydrolyzed by an enzymatic activity found in a physiologically-protected site, such as the brain and central nervous system and more particularly including neuronal, glial and other brain cell types, wherein said enzymatic activity is preferably an esterase and most preferably an esterase having a differential expression and activity profile in different tissue cell types. In additional preferred embodiments, specific release of the psychotropic, neurotropic or neurological drug or agent of the invention is achieved by enzymatic or chemical release of these drugs by extracellular cleavage of a cleavable linker moiety *via* an enzymatic activity specific for, *for example*, brain tissue, followed by specific uptake of the released psychotropic, neurotropic or neurological drug or agent by the appropriate cell in said tissue.

In another embodiment of this aspect of the invention, the spacer molecule is a peptide of formula $(\text{amino acid})_n$, wherein n is an integer between 2 and 25, preferably wherein the peptide comprises a polymer of one or more amino acids.

In still further embodiments of the compositions of matter of the invention are provided psychotropic, neurotropic or neurological drugs or agents having a first functional linker group, and a polar lipid carrier having a second functional linker group, wherein the drug is covalently linked directly to the polar lipid carrier by a chemical bond between the first and second functional linker groups. In preferred embodiments, each of the first and second functional linker groups is a hydroxyl group, a primary or secondary amino group, a phosphate group or substituted derivatives thereof or a carboxylic acid group. Preferred psychotropic, neurotropic or neurological drugs or

agents comprising the conjugates of the invention include L-dopa, hydroxytryptamine and metabolites thereof; amantadine, benztropine, bromocryptine, diphenhydramine, levadopa (a particularly preferred embodiment) and combinations thereof (*e.g.*, with carbidopa as provided as Sinemet®); pergolid, trihexphenidyl, ethosuximide, valproic acid, carbamazepine (*e.g.*, Tegretol®) and, in a particularly preferred embodiment, the 10- or 11-hydroxy analogues of carbamazepine; primidone, gabapentin in a particularly preferred embodiment; lamotrigine in a particularly preferred embodiment; felbamate, paramethadione and trimethadione; phenothiazines, thioxanthemes and related compounds; clozapine, haldoperidol, loxapine (Loxitane®), benzodiazapene antidepressants of the norepinephrine reuptake inhibitor type; monoamine oxidase inhibitors; anticonvulsants and antiepileptic agents, such as phenytoin (*e.g.*, Dilantin, Dilabid, or Zentropil); and antioxidants such as carotenes, glutathione and N-acetylcysteine. Preferred polar lipids include but are not limited to acyl- and acylated carnitine, sphingosine, ceramide, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, cardiolipin and phosphatidic acid. Pharmaceutical compositions comprising the drug/polar lipid conjugates of the invention are also provided.

Preferred embodiments of this aspect of the invention include compositions of matter that are polar lipid conjugates of anticonvulsive agents, antiepileptic agents, antiparkinsonian drugs, alkaloids, catecholamines including dopamine analogues and derivatives, muscarinic receptor agonists and antagonists, cholinergic receptor agonists and antagonists, calcium channel blockers, γ-aminobutyric acid (GABA) receptor agonists, antagonists, and uptake inhibitors and enhancers; phenothiazines, thioxanthemes and related compounds; clozapine, haldoperidol, loxapine (Loxitane®), benzodiazapene antidepressants of the norepinephrine reuptake inhibitor type; monoamine oxidase inhibitors; antidepressants and antimanic agents, antioxidants and other compounds that mitigate the effects of reactive oxygen species (for the treatment of Alzheimer's disease, Parkinson's disease, or other neurodegenerative conditions such as ataxia telangiectasia and amyelolatcrosclerosis (ALS)).

As disclosed herein, the invention comprehends a polar lipid-drug conjugate wherein the polar lipid selectively promotes association with and transit across certain physiological barriers to protected tissue sites, thereby facilitating delivery of drugs and other pharmaceutical agents to such physiologically restricted or protected sites. In

embodiments comprising a spacer moiety, the spacer component of the conjugates of the invention will preferably act to specifically release the drug from the lipid at the target site; prevent the non-specific release from the drug from the lipid in the systemic circulation or in hepatic, renal or other inappropriate cells, tissue or organs; target the conjugate to a specific cell or cell type within the protected tissue; prevent interaction and/or uptake of the drug by hematopoietic, ocular, hepatic or renal tissues; or perform other functions to maximize the effectiveness of the drug.

This type of conjugate has numerous advantages. The drug-lipid conjugates of the invention provide delivery of a variety of psychotropic, neurotropic and neurological drugs and agents to physiologically restricted or protected sites *in vivo* at concentrations and pharmicokinetic rates not heretofore attainable. A benefit of this advantage is the achievement of therapeutic indices of agents in such protected sites whereby the agent is useful for achieving a desired therapeutic goal. Another benefit is decreased hepatic toxicity, hematopoietic suppression (such as thrombocytopenia, leukopenia, aplastic anemia, leukocytosis, eosinophilia, pancytopenia, agranulocytosis), reduced systemic metabolism, degradation and toxicity, reduced hepatic clearance, reduced systemic adverse drug interactions, and generally reduced side effects due to the achievement of a lower, therapeutically-effective dose as the result of surmounting the physiological barrier. These biological effects can also result in simplified dosage schedules, particularly for drugs with short systemic half-lives.

In addition, the lipid/drug conjugates promote the intracellular entry of a variety of potentially useful drugs at pharmicokinetic rates not currently attainable. The range of targeted cell types is not limited *per se* by particular, limited biological properties of the cell (such as the number and type of specific receptor molecules expressed on the cell surface). In contrast to traditional attempts to simply target drugs to specific cells, the conjugates of the invention can also target drugs to specific intracellular organelles and other intracellular compartments. In certain preferred embodiment, the conjugates of the invention incorporate a variable spacer region that allow pharmacologically-relevant rates of drug release from polar lipid carrier molecules to be engineered into the compositions of the invention, thereby increasing their clinical efficacy and usefulness. Thus, time-dependent drug release and specific drug release in cells expressing the appropriate degradative enzymes are a unique possibility using the drug-lipid conjugates of the invention.

In particular, felicitous design of the psychotropic, neurotropic/neurological drug/spacer/polar lipid conjugate can provide an *in vivo* reservoir of time-dependent drug release in the physiologically protected tissue, resulting in specific delivery of therapeutic amounts to such tissues using a reduced dosage regime to minimize non-specific, systemic and deleterious side effects. In such formulations, the amount and activity of the psychotropic, neurotropic or neurological drug can be modulated by release *via* cleavage, preferably hydrolytic cleavage, of the spacer moiety, most preferably by an enzymatic activity in the protected tissue (*e.g.*, brain) that has a differential pattern of expression or activity in different cell types in said tissue. The conjugates of the invention can also be combined with other drug delivery approaches to further increase specificity and to take advantage of useful advances in the art.

Specific preferred embodiments of the present invention will become evident from the following more detailed description of certain preferred embodiments and the claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 depicts the synthetic scheme put forth in Example 1.

Figure 2 depicts the synthetic scheme put forth in Example 2.

Figure 3 depicts the synthetic scheme put forth in Example 3.

Figure 4 depicts the synthetic scheme put forth in Example 4.

Figure 5 depicts the synthetic scheme put forth in Example 5.

Figures 6A through 6D depict prodrugs tested as in Example 6.

Figure 7 depicts the synthetic scheme put forth in Example 7.

Figure 8 depicts the synthetic scheme put forth in Example 8.

Figure 9 depicts the synthetic scheme put forth in Example 9.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS

The present invention provides compositions of matter and methods for facilitating the entry into cells of biologically-active compounds. For the purposes of this invention, the term "biologically-active compound" is intended to encompass all naturally-occurring or synthetic compounds capable of eliciting a biological response or having an effect, either beneficial or cytotoxic, on biological systems, particularly cells and cellular organelles. These compounds are intended to include but are not

limited to all varieties of drugs, particularly psychotropic, neurotropic and neurologically-acting drugs and agents.

As used herein the terms "psychotropic, neurotropic and neurologically-acting drugs and agents" are intended to include any drug, agent or compound having a neurological, neurotropic, or psychotropic effect in an animal, preferably a human. These terms are intended to encompass anti-inflammatory agents, corticosteroids, sedatives, tranquilizers, narcotics, analgesics, anesthetics, antiepileptic, anticonvulsive and antispasmodic agents, antiparkinsonian drugs, alkaloids, catecholamines, including dopamine analogues and derivatives, muscarinic receptor agonists and antagonists, cholinergic receptor agonists and antagonists, calcium channel blockers, γ-aminobutyric acid (GABA) receptor agonists, antagonists, and uptake inhibitors and enhancers; phenothiazines, thioxanthemes and related compounds; clozapine, haldoperidol, loxapine (Loxitane®), benzodiazapene antidepressants of the norepinephrine reuptake inhibitor type; monoamine oxidase inhibitors; antidepressants and antimanic agents, antioxidants such as carotenes, glutathione, N-acetylcysteine or other molecules that mitigate the effects of reactive oxygen species for the treatment of Alzheimer's disease, Parkinson's disease, or other neurodegenerative conditions such as ataxia telangiectasia and amyelolaterosclerosis (ALS); neuroregenerative agents; and agents for the treatment of ischemia and other vascular diseases of the central nervous system. Appropriate formulations and pharmaceutical compositions of the neurotropic/neurological/ psychotropic drug/ polar lipid conjugates of the invention will be apparent and within the skill of one of ordinary skill in this art to advantageously prepare in view of the instant disclosure.

The compositions of matter provided by the invention comprise the biologically-active compounds of the invention covalently linked to a polar lipid carrier. A polar lipid carrier, as defined herein is intended to mean any polar lipid having an affinity for, or capable of crossing, a biological membrane and in particular a physiological barrier protecting certain cells, tissues and organs, including but not limited to sphingosine, ceramide, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, cardiolipin, phosphatidic acid, sphingomyelin and other sphingolipids, as these terms are understood in the art (*see*, Lehninger, *Biochemistry*, 2d ed., Chapters 11 & 24, Worth Publishers: New York, 1975). Also encompassed by the definition of "polar lipid" as used herein are "lyso" forms of

phosphatidyl embodiments of the invention (*i.e.*, lysophosphatidyl choline, lysophosphatidyl glycerol, lysophosphatidyl ethanolamine, lysophosphatidyl inositol, lysophosphatidyl serine, and lysophosphatidic acid), whereby one of the glycerol esterified fatty acid moieties comprising the polar lipid is removed and substituted with the conjugation of a drug or drug-conjugated spacer moiety (*see* Examples 8 and 9). Additionally, certain other lipids, such as acylated carnitine, comprise the conjugates of the invention (*see* Small, 1986, "From alkanes to phospholipids," *Handbook of Lipid Research: Physical Chemistry of Lipids*, Volume 4, Chapters 4 and 12, Plenum Press: New York). For the purposes of this invention, the term "polar lipid" is not intended to encompass "lipoid"-type compounds, such as, for example, aliphatic phosphonates (*see, for example*, U.S. Patent No. 5,413,996).

The compositions of matter of the invention may be further comprised of a spacer moiety comprising a first end and a second end, each end of the spacer having a functional linking group. For the purposes of this invention, the term "spacer" or "spacer moiety" is intended to encompass any chemical entity that links the biologically-active compound and the polar lipid. Such spacer moieties may be designed to facilitate the attachment of the conjugates of the invention to a target cell, or to facilitate, influence, modulate or regulate the release of the biologically-active compound at the desired target site. Such spacers may also facilitate enzymatic release at certain intracellular sites. Spacer groups, as described herein, include, but are not limited to aminohexanoic acid, polyglycine, polyamides, polyethylenes, and short functionalized polymers having a carbon backbone which is from one to about twelve carbon molecules in length. Particularly preferred embodiments of such spacer moieties comprise peptides of formula $(\text{amino acid})_n$, wherein n is an integer between 2 and 25 and the peptide is a polymer of one or more amino acids.

The term "linker functional group" is defined herein as any functional group for covalently binding the polar lipid carrier or biologically-active agent to the spacer group. These groups can be designated either "weak" or "strong" based on the stability of the covalent bond which the linker functional group will form between the spacer and either the polar lipid carrier or the biologically-active compound. The weak functionalities include, but are not limited to phosphoramide, phosphoester, carbonate, amide, carboxyl-phosphoryl anhydride, thioester and most preferably ester. The strong functionalities include, but are not limited to ether, thioether, amine, amide and most

preferably ester. The use of a strong linker functional group between the spacer group and the biologically-active compound will tend to decrease the rate at which the compound will be released at the target site, whereas the use of a weak linker functional group between the spacer group and the compound may act to facilitate release of the compound at the target site. Enzymatic release is, of course, also possible, but such enzyme-mediated modes of release will not necessarily be correlated with bond strength in such embodiments of the invention. Spacer moieties comprising enzyme active site recognition groups, such as spacer groups comprising peptides having proteolytic cleavage sites therein, are envisioned as being within the scope of the present invention. Specifically, such specifically-cleavable peptides are preferably prepared so as to be recognized by enzymes present in brain and other physiologically restricted or protected sites *in vivo*, so that the drug is preferentially liberated from the polar lipid conjugate at appropriate drug delivery sites. An illustrative example of such a specifically-cleavable peptide is a portion of the proopiomelanocortin family of peptides, which are cleaved in mammalian brain tissue to release a variety of peptides hormones and effector molecules, such as the enkephalins. Other beneficial and advantageous specifically-cleavable peptides will be recognized by those of ordinary skill in the art.

The drug/polar lipid conjugates of the invention are preferably provided comprised of spacer moieties that impart differential release properties on the conjugates related to differential expression or activity of enzymatic activities in physiologically restricted or protected sites in comparison with such activities in systemic circulation or in inappropriate targets, such as hepatic, renal or hematopoietic tissues. Differential release is also provided in certain embodiments in specific cell types comprising such physiologically protected tissues.

In particularly preferred embodiments of the present invention are provided psychotropic/neurotropic/neurological drug/polar lipid conjugates for specific delivery to brain tissue for the alleviation or amelioration of pathological disease states in the brain. Thus, the present invention provides methods and compositions of matter for facilitating the transit of such polar lipid conjugates of psychotropic, neurotropic or neurological drugs, agents and compounds across the blood-brain barrier and into targeted regions of the brain, for the treatment of animal, preferably human, diseases and pathological conditions. Among the most common such diseases and conditions are Alzheimer's disease, Parkinson's disease, epilepsy and other seizure disorders (such as

petit mal, grand mal, tonic-clonic seizure disorder, parietal complex seizure, and psychomotor seizures), migraine, neurodegenerative conditions such as ataxia telangiectasia and ALS, Lennox-Gastaut syndrome, neuropathy such as trigeminal neuralgia, diabetic neuropathy, shingles, and psychological disorders, including bipolar disorder, explosive aggression, depression and agitation associated with elderly dementia.

The invention provides polar lipid/drug conjugates comprising psychotropic, neurotropic and neurological drugs, agents and compounds including but not limited to L-dopa, hydroxytryptamine and metabolites thereof; amantadine, benztropine, bromocryptine, diphenhydramine, levadopa (a particularly preferred embodiment) and combinations thereof (*e.g.*, with carbidopa as provided as Sinemet®); pergolid, trihexphenidyl, ethosuximide, valproic acid, carbamazepine (*e.g.*, Tegretol®) and, in a particularly preferred embodiment, the 10- or 11-hydroxy analogues of carbamazepine; primidone, gabapentin in a particularly preferred embodiment; lamotrigine in a particularly preferred embodiment; felbamate, paramethadione and trimethadione; phenothiazines, thioxanthemes and related compounds; clozapine, haldoperidol, loxapine (Loxitane®), benzodiazapene antidepressants of the norepinephrine reuptake inhibitor type; monoamine oxidase inhibitors; anticonvulsants and antiepileptic agents, such as phenytoin (*e.g.*, Dilantin, Dilabid, or Zentropil); and antioxidants such as carotenes, glutathione and N-acetylcysteine.

The invention specifically provides methods for preparing and administering such psychotropic, neurotropic and neurological drugs, agent and compounds for use in treating pathological conditions *in vivo*.

Animals to be treated with the drug-polar lipid conjugates using the methods of the invention are intended to include all vertebrate animals, preferably domesticated animals, such as cattle, horses, goats, sheep, fowl, fish, household pets, and others, as well as wild animals, and most preferably humans.

The following Examples illustrate certain aspects of the above-described method and advantageous results. The following examples are shown by way of illustration and not by way of limitation.

EXAMPLE 1

A polar lipid conjugate with levadopa is prepared by conjugating a linker moiety

to a polar lipid *via* an amide linkage, as follows. A polar lipid (sphingosine) comprising unconjugated amino groups is reacted with a 6-hydroxyhexanoic acid (6-HHA) in the presence of 1.0 equivalent of dicyclohexyl carbodiimide (DCCD) overnight at 40-50°C. The derivatized sphingosine was then reacted with 0.1N methanolic potassium hydroxide at room temperature, and then treated with 2.0 equivalents of *tert*-butyl dimethyl silyl imidazole (TBDMS) overnight at 40-50°C. This sphingosine species, derivatized by an amide linkage between the amino group of sphingosine and the carboxylate group of 6-HHA, is then esterified at the unprotected 6-HHA derived hydroxyl group with *bis*-TBDMS-levadopa and DCCD overnight at 40-50°C. The levadopa-sphingosine conjugate is then deprotected by treatment with 4.0 equivalents of *t*-butylammonium fluoride at 0°C for 10 minutes. This reaction scheme is illustrated in Figure 1. Synthesis of conjugates comprising ester linkages as described in Examples 1, 2 and 3, and of amine linkages as described in Examples 4 and 5 advantageously permits control of rates of drug release based on differences in amount and rates of amidase or esterase enzymatic activity in the brain, wherein amidase-sensitive linkages generally provide a longer time release course than esterase-sensitive linkages.

EXAMPLE 2

A polar lipid conjugate with gabapentin comprising an ester linkage is prepared by conjugating a linker moiety to a polar lipid *via* an amide linkage, as follows. A polar lipid (sphingosine) comprising unconjugated amino groups is reacted with a 6-hydroxyhexanoic acid (6-HHA) in the presence of 1.0 equivalent of dicyclohexyl carbodiimide (DCCD) overnight at 40-50°C. The derivatized sphingosine was then reacted with 0.1N methanolic potassium hydroxide at room temperature, and then treated with 2.0 equivalents of *tert*-butyl dimethyl silyl imidazole (TBDMS) overnight at 40-50°C. This sphingosine species, derivatized by an amide linkage between the amino group of sphingosine and the carboxylate group of 6-HHA, is then esterified at the unprotected 6-HHA derived hydroxyl group with gabapentin and DCCD overnight at 40-50°C. The gabapentin-sphingosine conjugate is then deprotected by treatment with 4.0 equivalents of *t*-butylammonium fluoride at 0°C for 10 minutes. This reaction scheme is illustrated in Figure 2.

EXAMPLE 3

A polar lipid conjugate with 10-hydroxycarbamazepine comprising an ester linkage is prepared by conjugating a linker moiety to a polar lipid *via* an amide linkage, as follows. A polar lipid (sphingosine) comprising unconjugated amino groups is reacted with a activated adipic acid monomethyl ester (AMME) in the presence of 1.0 equivalent of dicyclohexyl carbodiimide (DCCD) overnight at 40-50°C. The derivatized sphingosine was then reacted with 0.1N methanolic potassium hydroxide at room temperature, and then treated with 2.0 equivalents of *tert*-butyl dimethyl silyl imidazole (TBDMS) overnight at 40-50°C. This sphingosine species, derivatized by an amide linkage between the amino group of sphingosine and the 1-carboxylate group of AMME is then esterified at the 7-carboxylate group of unprotected adipic acid with 10-hydroxycarbamazepine and DCCD overnight at 40-50°C. The 10-hydroxycarbamazepine-sphingosine conjugate is then deprotected by treatment with 4.0 equivalents of *t*-butylammonium fluoride at 0°C for 10 minutes. This reaction scheme is illustrated in Figure 3.

EXAMPLE 4

A polar lipid conjugate of gabapentin, hydroxycarbamazepine or levadopa is prepared by conjugating a specifically-cleavable peptide as a linker between a polar lipid and a drug as follows. An derivatized polar lipid comprising unconjugated amino groups is reacted with a proteolytically-inert peptide in which the terminal amine and any of the constituent amino acid sidechain reactive amines are covered by *tert*-butoxycarbonyl (*t*-Boc) protecting groups in the presence of triphenyl phosphine as described by Kishimoto (1975, *Chem. Phys. Lipids* 15: 33-36). The peptide/polar lipid conjugate is then reacted in the presence of pyridine hydrofluoride as described by Matsuura *et al.* (1976, *J. Chem. Soc. Chem. Comm.* pp. 451-459) to remove the *t*-Boc protecting groups. The peptide/polar lipid is then conjugated to the specifically-cleavable peptide, in which the terminal amine and any of the constituent amino acid sidechain reactive amines are covered by *t*-Boc protecting groups, as described in the presence of triphenyl phosphine. After deprotection of reactive amines with pyridine hydrofluoride as described, gabapentin, hydroxycarbamazepine or levadopa is conjugated to a free amino group of the polar lipid/peptide/specifically-cleavable peptide *via* a reactive carboxylic acid group to yield a drug/polar lipid conjugate of the

invention. This reaction scheme is illustrated in Figure 4 for sphingosine conjugated to levadopa.

EXAMPLE 5

Carbamazepine is directly conjugated to sphingosine *via* an amide linkage as follows. Sphingosine is reacted with 1,3 *bis*(trimethylsilyl)urea as described by Verbloom *et al.* (1981, *Synthesis* 1032: 807-809) to give a trimethylsilyl derivative of sphingosine. The sphingosine derivative is then conjugated with carbamazepine in the presence of triphenylphosphine as described by Kishimoto (*Ibid.*). The sphingosine-carbamazepine conjugate is then reacted in the presence of pyridine hydrofluoride as described by Matsuura *et al.* (*Ibid.*) to remove the *t*-Boc protecting group, to yield the drug/sphingosine conjugate covalently linked through an amide bond. This reaction scheme is illustrated in Figure 5.

EXAMPLE 6

The effect of presenting a biologically active compound such as a drug to mammalian cells as a prodrug covalently linked to a polar lipid carrier moiety was determined as follows. The antifolate drug methotrexate was conjugated with a variety of polar lipid carriers *via* organic spacer moieties having specific reactive functional groups. A representative sample of such compounds is shown in Figures 6A through 6C, wherein MC represents Mtx linked to sphingosine *via* an amide bond to a 6-aminohexanoic acid spacer, ME_nC represents Mtx linked to sphingosine *via* an ester linkage to a 6-hydroxyhexanoic acid spacer, and MSC represents Mtx linked to sphingosine *via* a salicylic acid ester linkage to a 6-aminohexanoic acid spacer. Also studied was a conjugate of azidothymidine linked to sphingosine *via* an ester linkage to a 6-hydroxyhexanoic acid spacer (N-AZT-ceramide; Figure 6D). The compounds were tested for their growth inhibitory effects on murine NIH 3T3 cells growing in cell culture. About one million such cells per P100 tissue culture plate were grown in DMEM media supplemented with 10% fetal calf serum (GIBCO, Grand Island, NY) in the presence or absence of a growth-inhibitory equivalent of each prodrug. Cell numbers were determined after 70 hours growth in the presence or absence of the prodrug. In a second set of experiments was included in the growth media an amount of a brain homogenate containing an enzymatically-active esterase.

The results from these experiments are shown in Table I. As can be seen from these data, the MC prodrug had no effect on the growth and survival of the cells. This result did not change upon co-incubation with the esterase-containing brain extract, which was expected due to the nature of the drug/spacer linkage (an amide bond). A different result was obtained with the ME_6C conjugate. The prodrug was ineffective in inhibiting cell growth or survival in the absence of brain extract. Upon addition of the brain extract, a significant increase in Mtx cytotoxicity was observed. This is consistent with cleavage of the ester linkage by the brain extract-derived esterase. A similar result was obtained with the MCS conjugate, indicating that the brain extract esterase activity was capable of cleaving the salicylic acid ester.

Table II shows the results of drug uptake studies performed with the prodrug N-AZT-ceramide. Antiviral amounts of the prodrug conjugate were added to NIH 3T3 cell cultures, and the antiviral activity of the prodrug was found to be equivalent to the activity of free AZT. In addition, upon removal of the prodrug, intracellular retention of prodrug was found to be up to 15-fold higher than free AZT (Table II) over a 23h period.

These results indicate that for Mtx-containing conjugates, the free drug must be released from the prodrug for biological activity. These results suggest that specific release of this drug, and perhaps others, can be achieved using cleavable linker moieties that are specifically cleaved only in pathogen-infected cells.

TABLE I

Sample[1]	# cells/plate[2]	Sample[3]	# cells/plate[4]
Control/FBS	7.8×10^6	Control/FBS	13×10^6
ME_6C/FBS	6.5×10^6	MSC/FBS	2.1×10^6
ME_6C/brain	2.7×10^6	MSC/brain	0.51×10^6
Mtx/FBS	0.16×10^6	Mtx/FBS	0.13×10^6
Mtx/brain	0.09×10^6	Mtx/brain	0.06×10^6
Control/brain	N.D.	Control/brain	6.2×10^6

[1] = cells incubated with drug/FBS or drug/brain extract for 1 hour at 37°C

[2] = cell growth and survival determined 70 hours after drug addition

[3] = cells incubated with drug/FBS or drug/brain extract for 2 hours at 37°C

[4] = cell growth and survival determined 72 hours after drug addition

5

TABLE II

Time[1]	AZT[2]	N-AZT-Ceramide[2]
0 hr.	6.49	8.45
23 hr.	0.55	7.78

10

[1] = time between the end of drug treatment and assay for intracellular drug concentration

15

[2] = nM/10^6 cells

EXAMPLE 7

A neurological agent of the invention is prepared wherein the drug levadopa (LDP) is conjugated to sphingosine *via* a 6-aminocaproic acid spacer. This reaction scheme is illustrated in Figure 7. The primary amino and hydroxyl groups of sphingosine are acylated by reaction with activated *N*-(levadopa)aminocaproic acid overnight at 40-50°C, followed by base hydrolysis in 0.1N methanolic KOH. The LDP derivative of 6-aminocaproic acid is synthesized by activating the carboxylic acid moiety of LDP and reacting with 6-aminocaproic acid for 2 days at 60-70°C. This reaction is stopped under acidic conditions to liberate anhydrides that form under these conditions.

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EXAMPLE 8

A neurological agent of the invention is prepared wherein the drug levadopa (LDP) is conjugated to lysophosphatidylethanolamine *via* a 6-aminocaproic acid spacer. This reaction scheme is illustrated in Figure 8. The free hydroxyl group of lysophosphatidylethanolamine are acylated by reaction with activated *N*-(levadopa)aminocaproic acid overnight at 40-50°C at pH 6.5-7.5, followed by base hydrolysis in 0.1N methanolic KOH. *N*-(levadopa)aminocaproyl-conjugated lysophosphatidylethanolamine is purified from side products of the synthetic reaction

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using conventional chemical purification techniques.

The LDP derivative of 6-aminocaproic acid is synthesized by activating the carboxylic acid moiety of LDP and reacting with 6-aminocaproic acid for 2 days at 60-70°C. This reaction is stopped under acidic conditions to liberate anhydrides that form under these conditions.

Lysophosphatidylethanolamine is commercially available, and can also be prepared from phsophatidylethanolamine by methanolic base hydrolysis in the presence of a cognate fatty alcohol to promote trans-esterification.

EXAMPLE 9

A polar lipid conjugate with 10-hydroxycarbamazepine is prepared by conjugating a linker moiety to a polar lipid *via* an ester linkage, as follows. A polar lipid (lysophosphatidylethanolamine) comprising unesterified hydroxyl groups is reacted with an activated adipic acid monomethylester (AMME) in the presence of 1.0 equivalent of dicyclohexyl carbodiimide (DCCD) overnight at 40-50°C at pH 6.5-7.5. The derivatized lysophosphatidylethanolamine was then reacted with 0.1N methanolic potassium hydroxide at room temperature, and optionally then treated with 2.0 equivalents of *tert*-butyl dimethyl silyl imidazole (TBDMS) overnight at 40-50°C.

This lysophosphatidylethanolamine species, derivatized by an ester linkage between the glycerol hydroxyl group of lysophosphatidylethanolamine and the 1-carboxylate group of adipic acid, is then esterified at the 7-carboxylate group of the adipic acid spacer with 10-hydroxycarbamazepine and DCCD overnight at 40-50°C. The 10-hydroxycarbamazepine-phosphatidylethanolamine conjugate is then deprotected, if appropriate, by treatment with 4.0 equivalents of *t*-butylammonium fluoride at 0°C for 10 minutes. This reaction scheme is illustrated in Figure 9, wherein the TBDMS protection step is not shown. Side products of the synthetic reaction are removed using conventional chemical purification techniques.

It should be understood that the foregoing disclosure emphasizes certain specific embodiments of the invention and that all modifications or alternatives equivalent thereto are within the spirit and scope of the invention as set forth in the appended claims.

What is claimed is:

1. A pharmaceutical composition comprising a psychotropic, neurotropic or neurological drug, a polar lipid carrier, two linker functional groups and a spacer, wherein the spacer has a first end and a second end and wherein the polar lipid is attached to the first end of the spacer through a first linker functional group and the drug is attached to the second end of the spacer through a second linker functional group.

2. The pharmaceutical composition of Claim 1 wherein the drug is L-dopa, hydroxytryptamine, amantadine, benztropine, bromocryptine, diphenhydramine, levadopa, phenytoin, pergolid, trihexphenidyl, ethosuximide, valproic acid, carbamazepine, 10-hydroxycarbamazepine, 11-hydroxycarbamazepine, primidone, gabapentin, lamotrigine, felbamate, paramethadione, trimethadione, phenothiazine, thioxantheme, clozapine, haldoperidol, loxapine, a benzodiazapene antidepressants of the norepinephrine reuptake inhibitor type, a monoamine oxidase inhibitor, phenytoin, carotene, glutathione or N-acetylcysteine.

3. A pharmaceutical composition according to Claim 1 wherein the spacer allows the drug to act without being released at an intracellular site and wherein the first linker functional group attached to the first end of the spacer is strong and the second linker functional group attached to the second end of the spacer is weak.

4. A pharmaceutical composition according to Claim 1 wherein the spacer allows the facilitated hydrolytic release of the drug at an intracellular site and wherein the first linker functional group attached to the first end of the spacer is strong and the second linker functional group attached to the second end of the spacer is weak.

5. A pharmaceutical composition according to Claim 1 wherein the spacer allows the facilitated enzymatic release of the drug at an intracellular site and wherein the first linker functional group attached to the first end of the spacer is strong and the second linker functional group attached to the second end of the spacer is weak.

6. A pharmaceutical composition according to Claim 1 wherein the polar lipid is acyl carnitine, acylated carnitine, sphingosine, ceramide, phosphatidyl choline,

phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, cardiolipin or phosphatidic acid.

7. A pharmaceutical composition comprising a psychotropic, neurotropic or neurological drug having a first functional linker group, and a polar lipid carrier having a second functional linker group, wherein the drug is covalently linked to the polar lipid carrier by a chemical bond between the first and second functional linker groups.

8. A pharmaceutical composition according to Claim 7 wherein the first functional linker group is a hydroxyl group, a primary or secondary amino group, a phosphate group or substituted derivatives thereof or a carboxylic acid group.

9. A pharmaceutical composition according to Claim 7 wherein the second functional linker group is a hydroxyl group, a primary or secondary amino group, a phosphate group or substituted derivatives thereof or a carboxylic acid group.

10. A pharmaceutical composition according to Claim 7 wherein the polar lipid is acyl carnitine, acylated carnitine, sphingosine, ceramide, phosphatidyl choline, phosphatidyl glycerol, phosphatidyl ethanolamine, phosphatidyl inositol, phosphatidyl serine, cardiolipin or phosphatidic acid.

11. The pharmaceutical composition of Claim 7 wherein the drug is L-dopa, hydroxytryptamine, amantadine, benztropine, bromocryptine, diphenhydramine, levadopa, phenytoin, pergolid, trihexphenidyl, ethosuximide, valproic acid, carbamazepine, 10-hydroxycarbamazepine, 11-hydroxycarbamazepine, primidone, gabapentin, lamotrigine, felbamate, paramethadione, trimethadione, phenothiazine, thioxantheme, clozapine, haldoperidol, loxapine, a benzodiazapene antidepressants of the norepinephrine reuptake inhibitor type, a monoamine oxidase inhibitor, phenytoin, carotene, glutathione or N-acetylcysteine.

12. Use of a pharmaceutical composition of Claim 1 for preparing a medicament in an acceptable carrier or formulation for treating a pathological condition

or disease state in cells, tissues or organs in an animal.

13. Use of a pharmaceutical composition of Claim 1 for preparing a medicament in an acceptable carrier or formulation for treating a pathological condition or disease state in an animal, wherein the pathological condition or disease state is present in a physiologically restricted or protected site in the animal.

14. A use according to Claim 12 or 13 wherein the animal is a human.

15. Use of a pharmaceutical composition of Claim 7 for preparing a medicament in an acceptable carrier or formulation for treating a pathological condition or disease state in cells, tissues or organs in an animal.

16. Use of a pharmaceutical composition of Claim 7 for preparing a medicament in an acceptable carrier or formulation for treating a pathological condition or disease state in an animal, wherein the pathological condition or disease state is present in a physiologically restricted or protected site in the animal.

17. A use according to Claim 15 or 16 wherein the animal is a human.

18. A pharmaceutical composition according to Claims 1 or 7 wherein the spacer is a peptide of formula $(\text{amino acid})_n$, wherein n is an integer between 2 and 25, and the peptide comprises a polymer of one or more amino acids.

19. A pharmaceutical composition according to Claim 1 comprising L-dopa, hydroxytryptamine, amantadine, benztropine, bromocryptine, diphenhydramine, levadopa, phenytoin, pergolid, trihexphenidyl, ethosuximide, valproic acid, carbamazepine, 10-hydroxycarbamazepine, 11-hydroxycarbamazepine, primidone, gabapentin, lamotrigine, felbamate, paramethadione, trimethadione, phenothiazine, thioxantheme, clozapine, haldoperidol, loxapine, a benzodiazapene antidepressants of the norepinephrine reuptake inhibitor type, a monoamine oxidase inhibitor, phenytoin, carotene, glutathione or N-acetylcysteine.

20. A use according to Claim 12, 13, 14, 15, 16 or 17, wherein the disease state is a neurological disease.

21. A use according to Claim 20 wherein the neurological disease is Alzheimer's disease, Parkinson's disease, epilepsy, seizure disorder, migraine or Lennox-Gastaut syndrome.

22. A use according to Claim 12, 13, 14, 15, 16 or 17, wherein the disease state is a neuropathy.

23. A use according to Claim 22 wherein the neuropathy is trigeminal neuralgia, diabetic neuropathy or shingles.

24. A use according to Claim 12, 13, 14, 15, 16 or 17, wherein the disease state is a psychological disorder.

25. A use according to Claim 24 wherein the psychological disorder is bipolar disorder, explosive aggression, depression or agitation associated with elderly dementia.

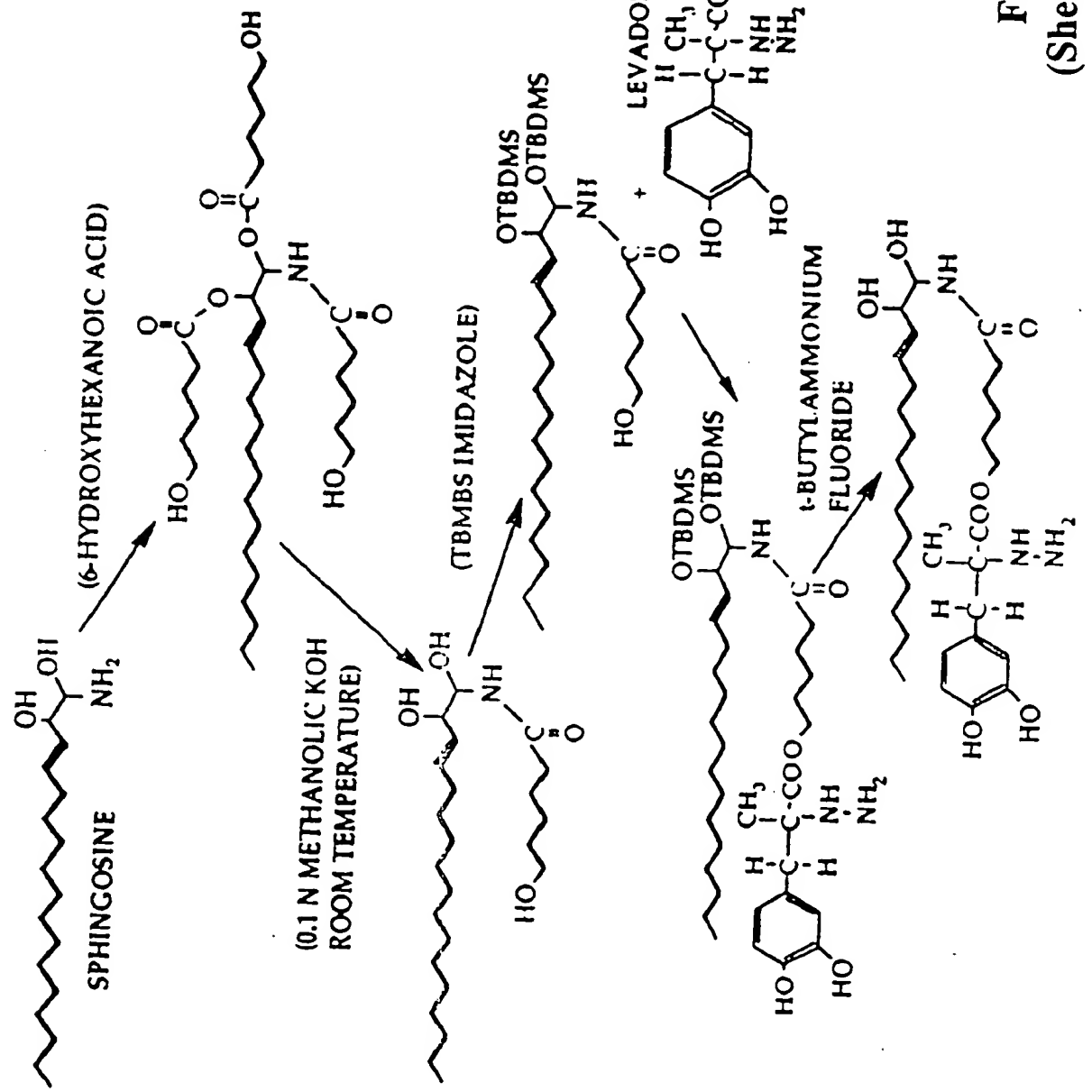


Figure 1
(Sheet 1 of 12)

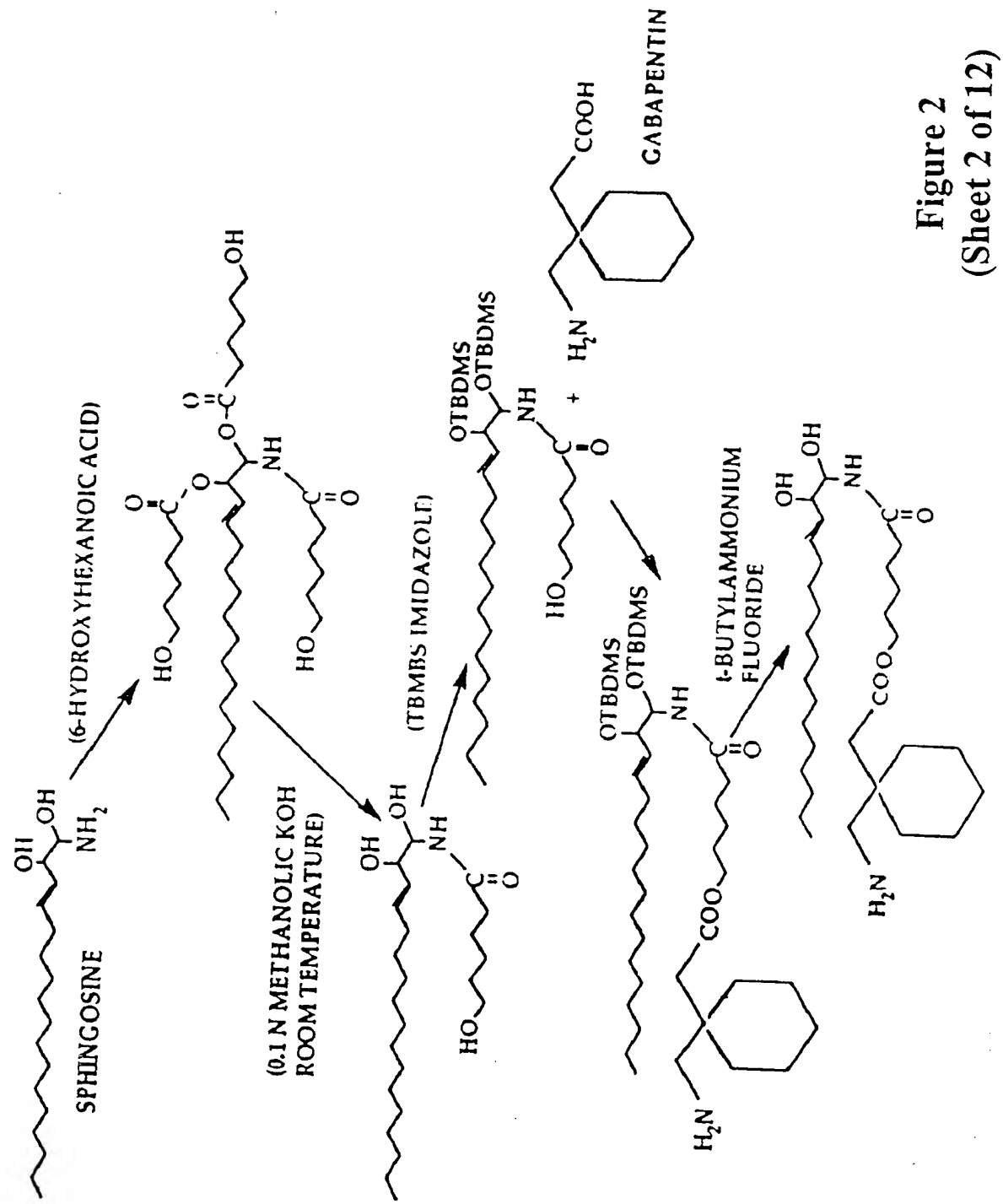


Figure 2
(Sheet 2 of 12)

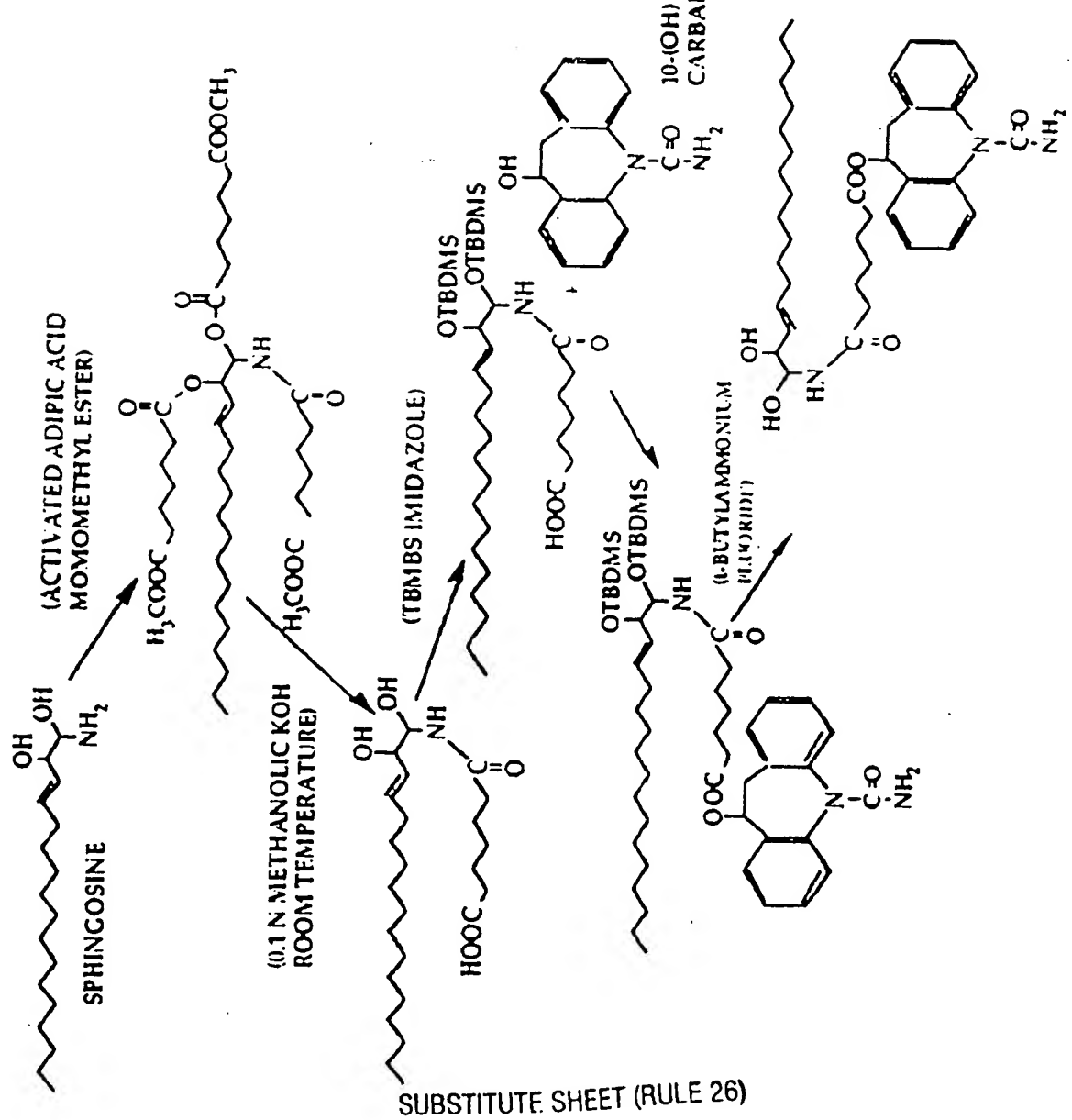
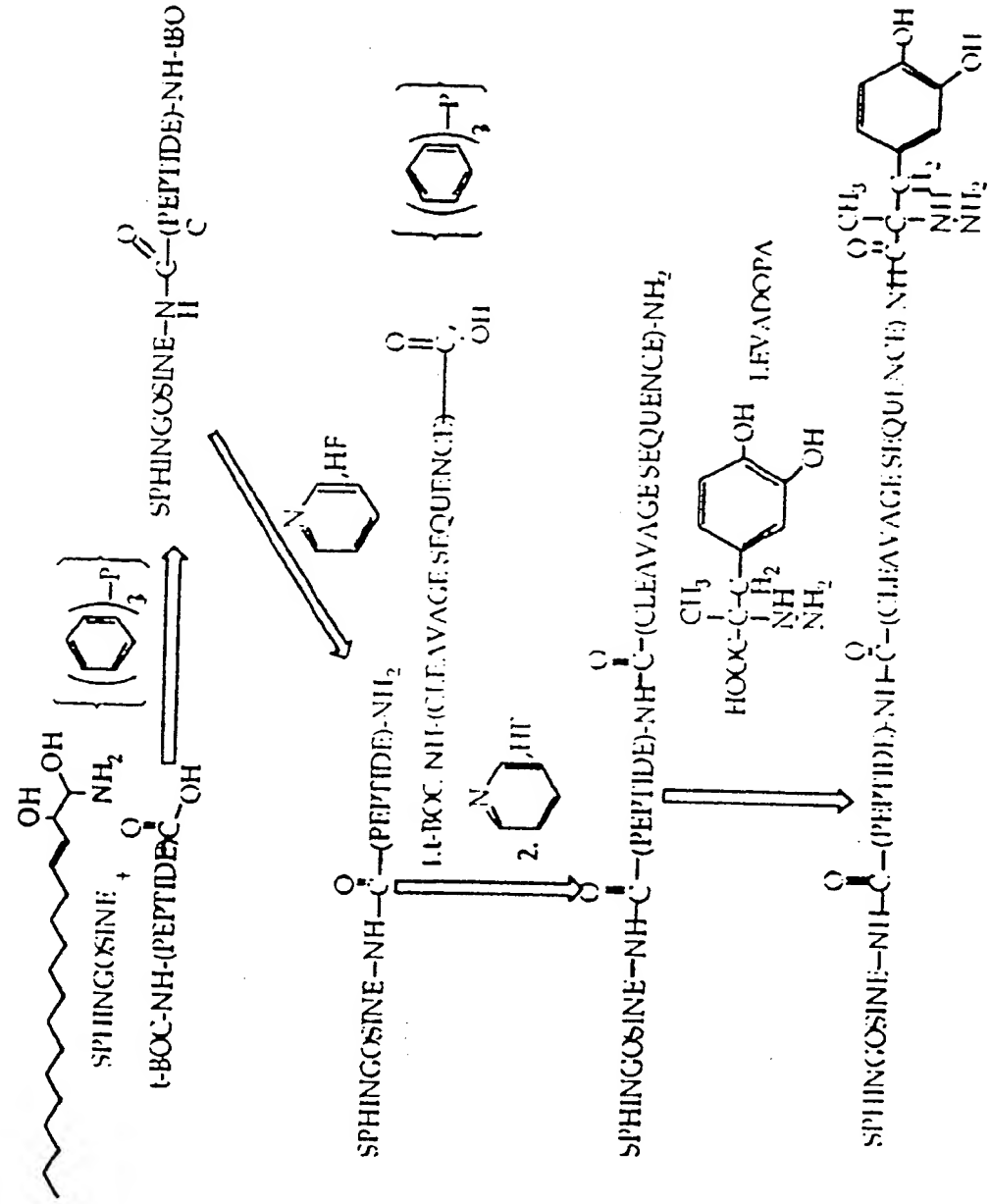


Figure 3
(Sheet 3 of 12)

Figure 4
(Sheet 4 of 12)



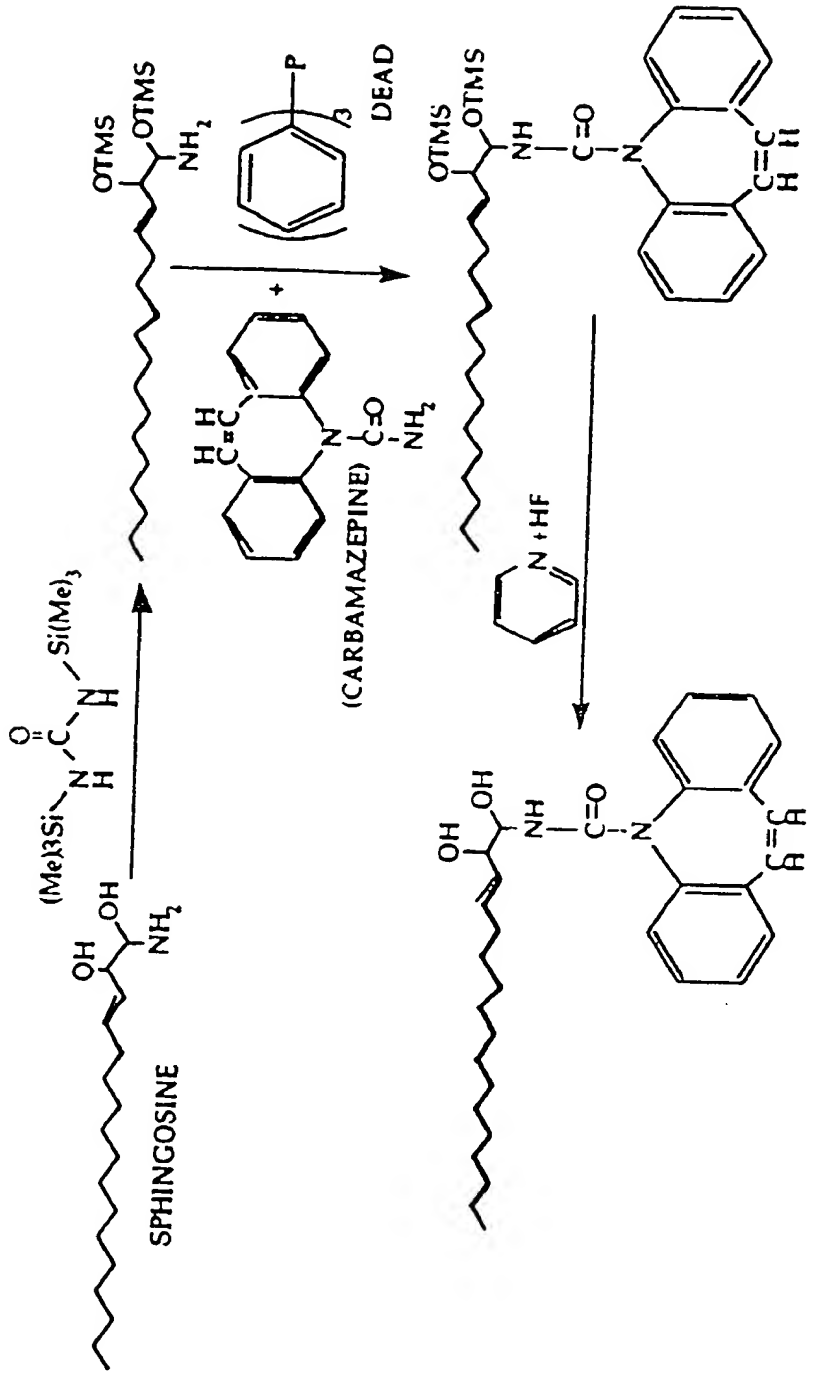


Figure 5
(Sheet 5 of 12)

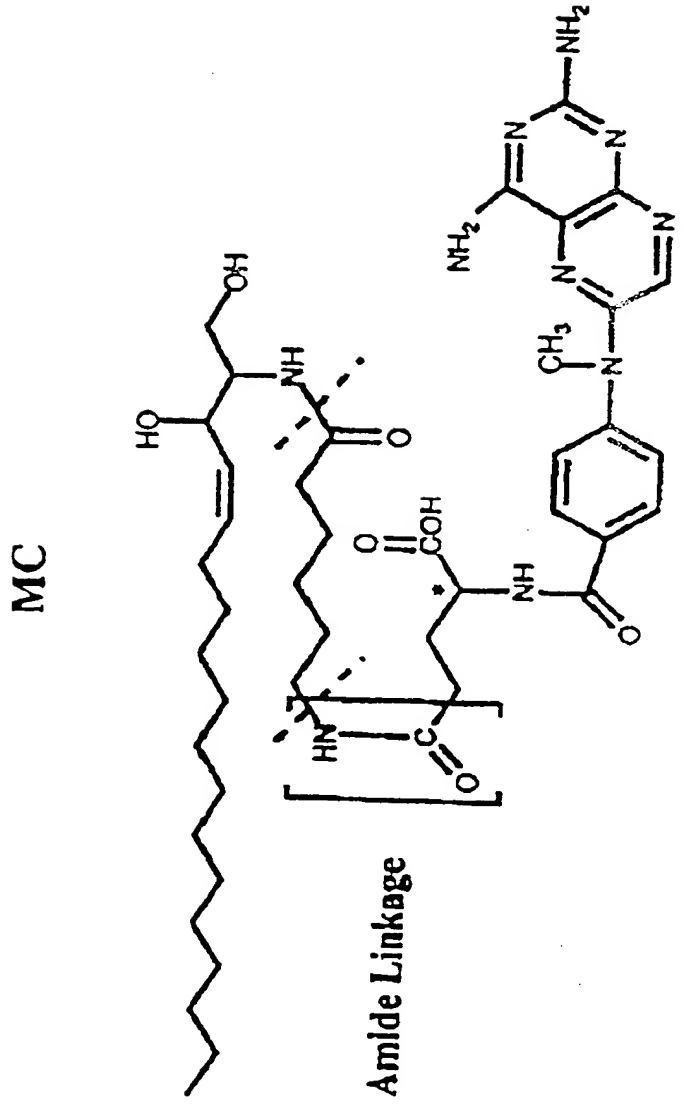


Figure 6A
(Sheet 6 of 12)

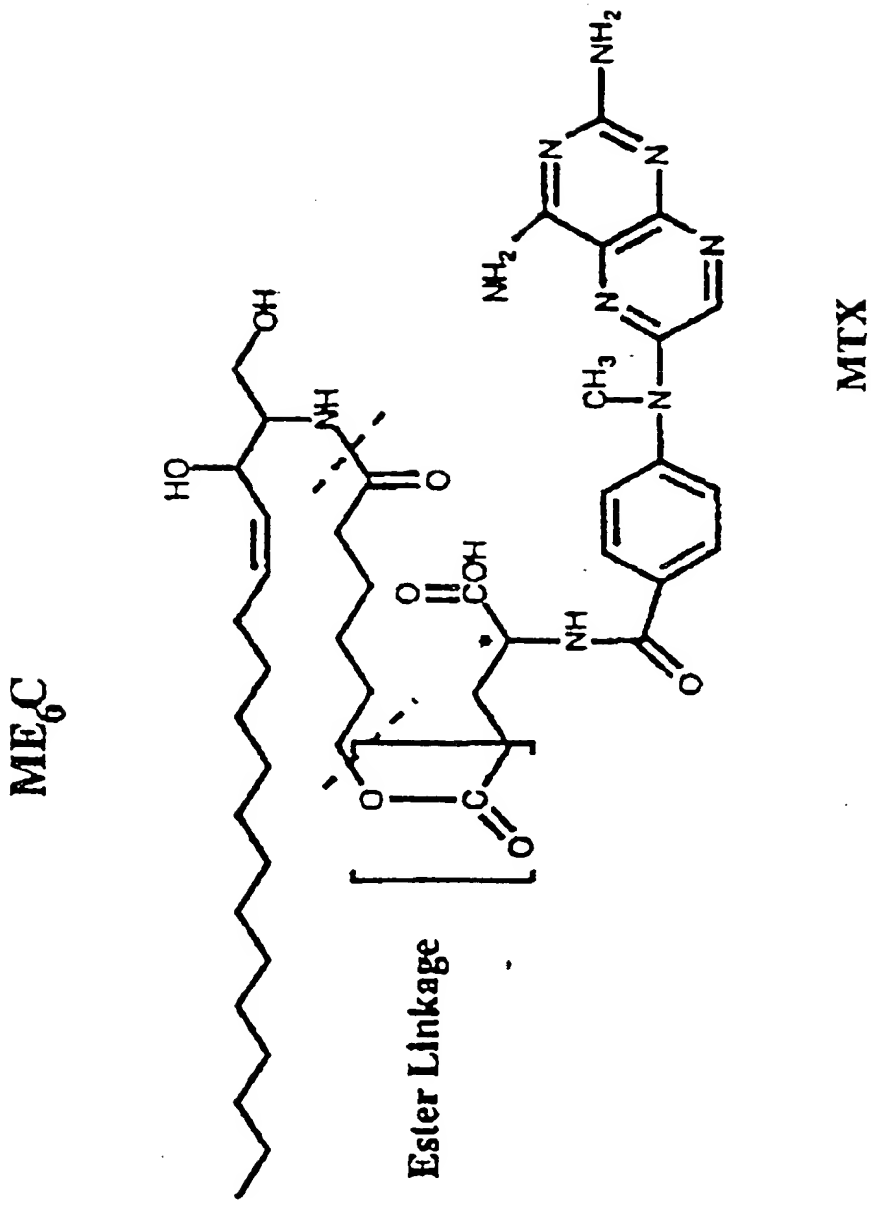


Figure 6B
(Sheet 7 of 12)

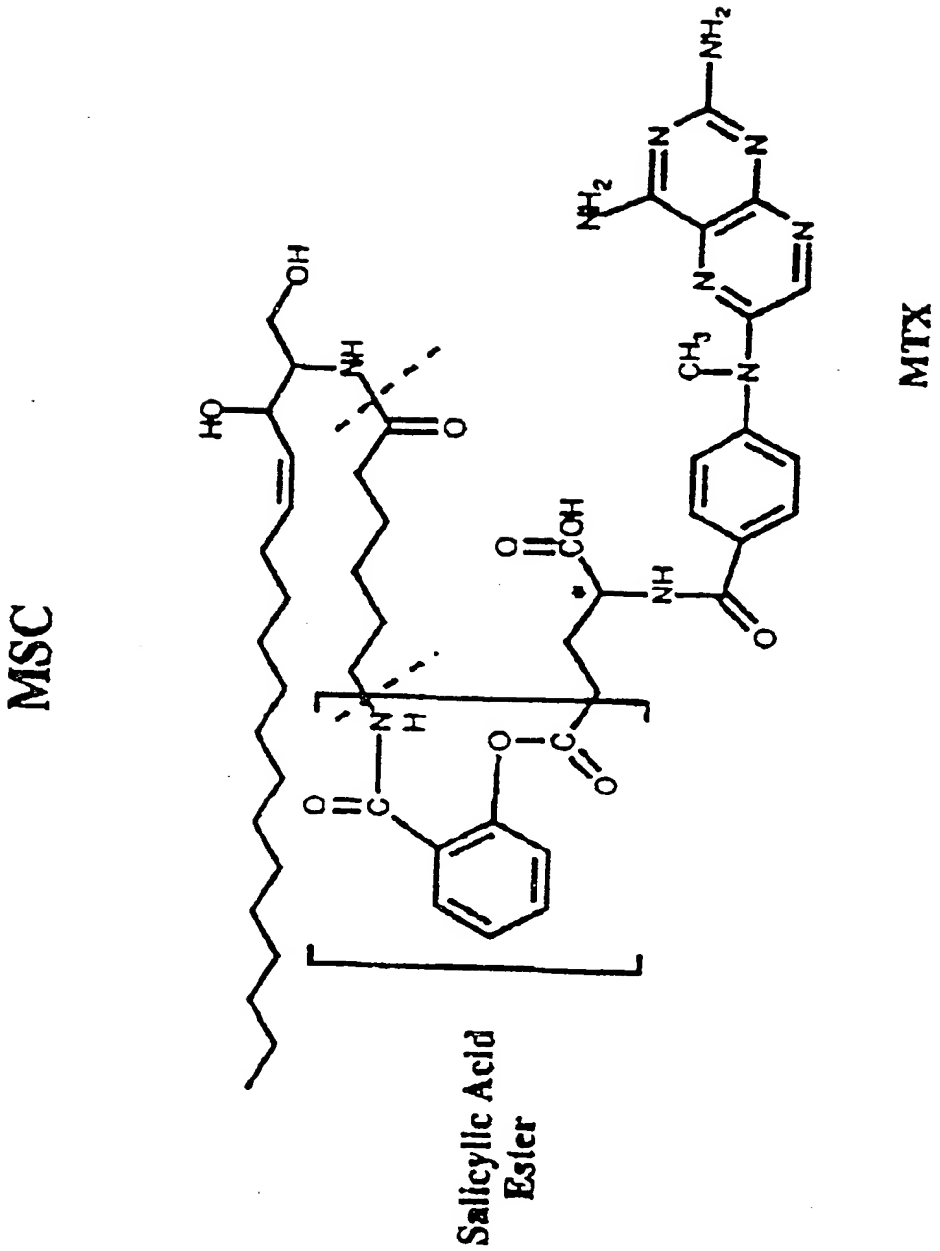


Figure 6C
(Sheet 8 of 12)

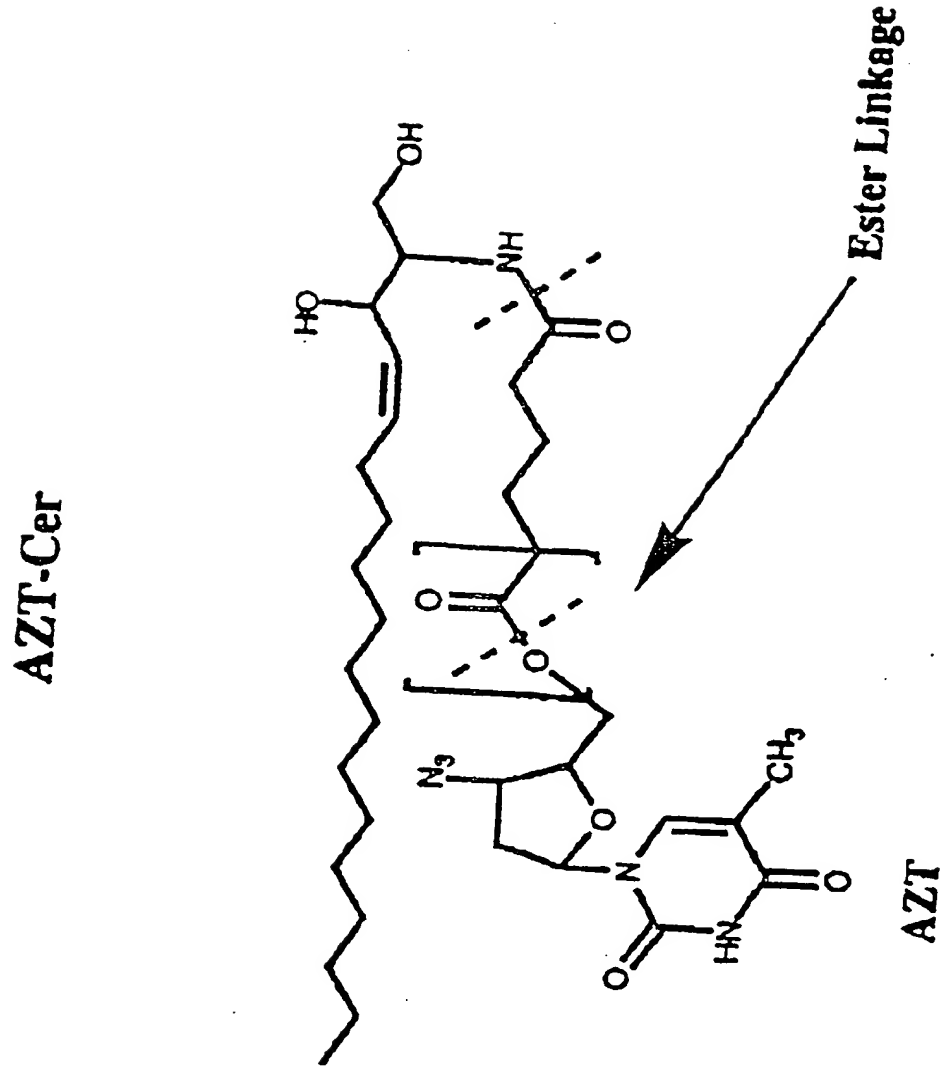


Figure 6D
(Sheet 9 of 12)

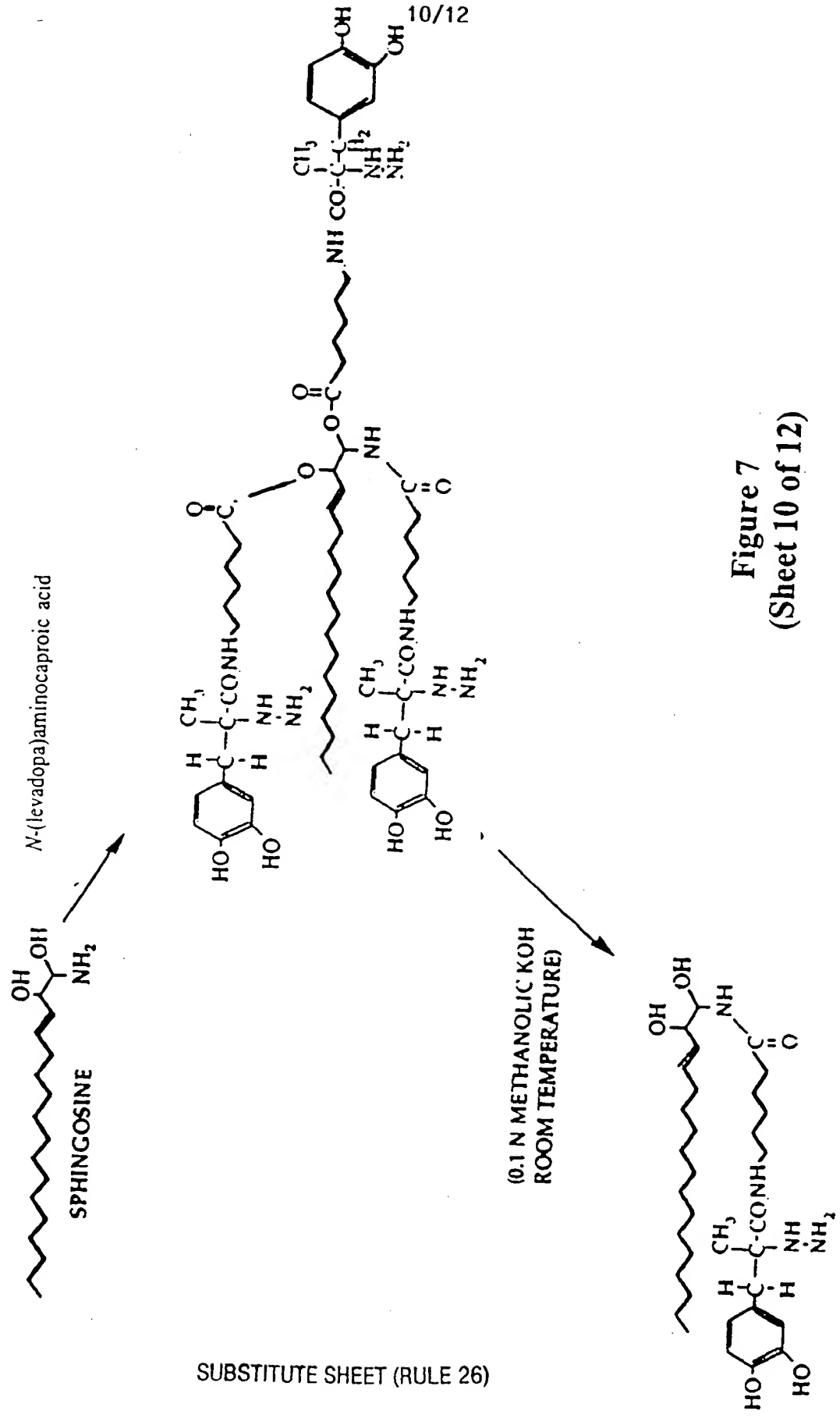


Figure 7
(Sheet 10 of 12)

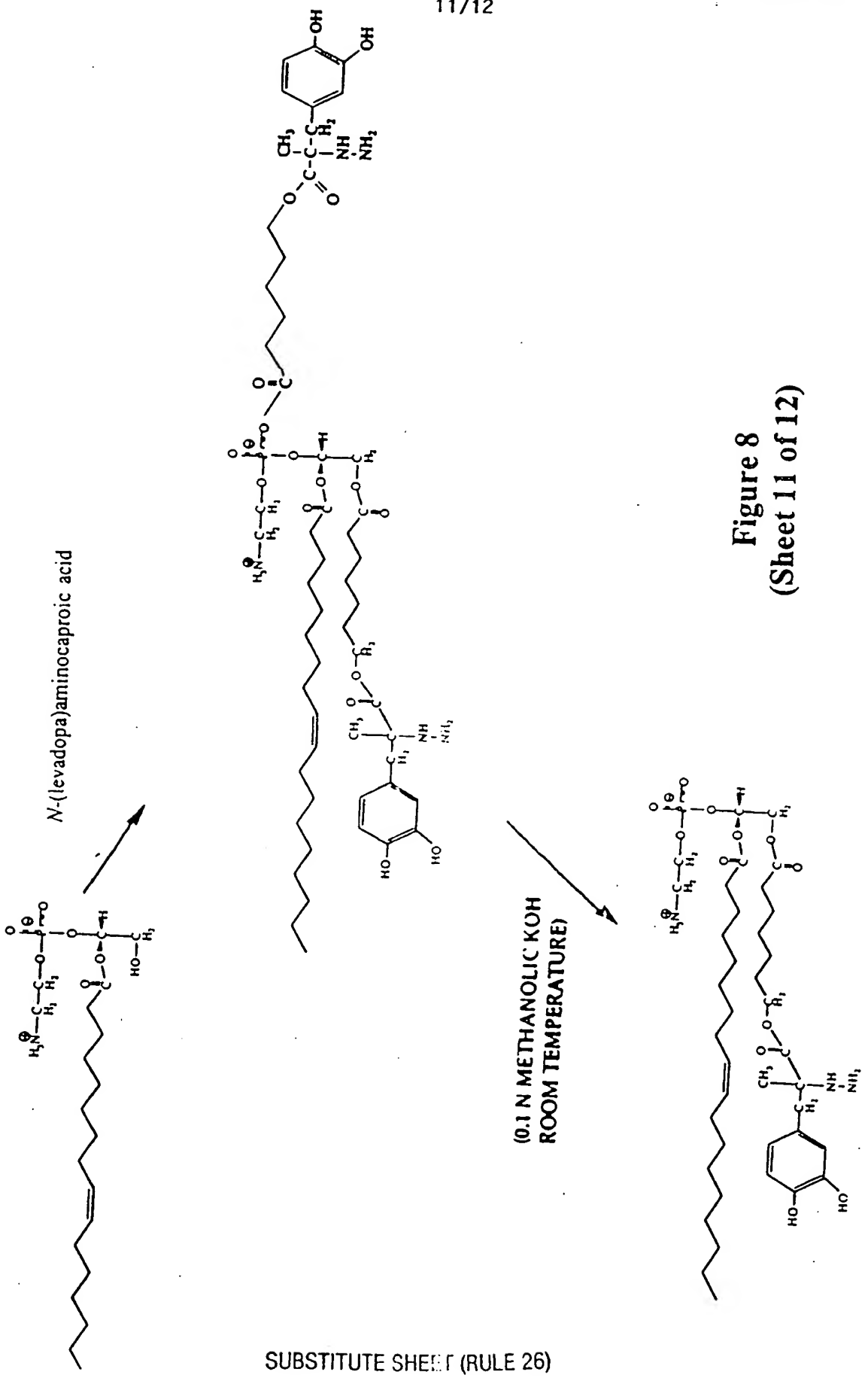


Figure 8
(Sheet 11 of 12)

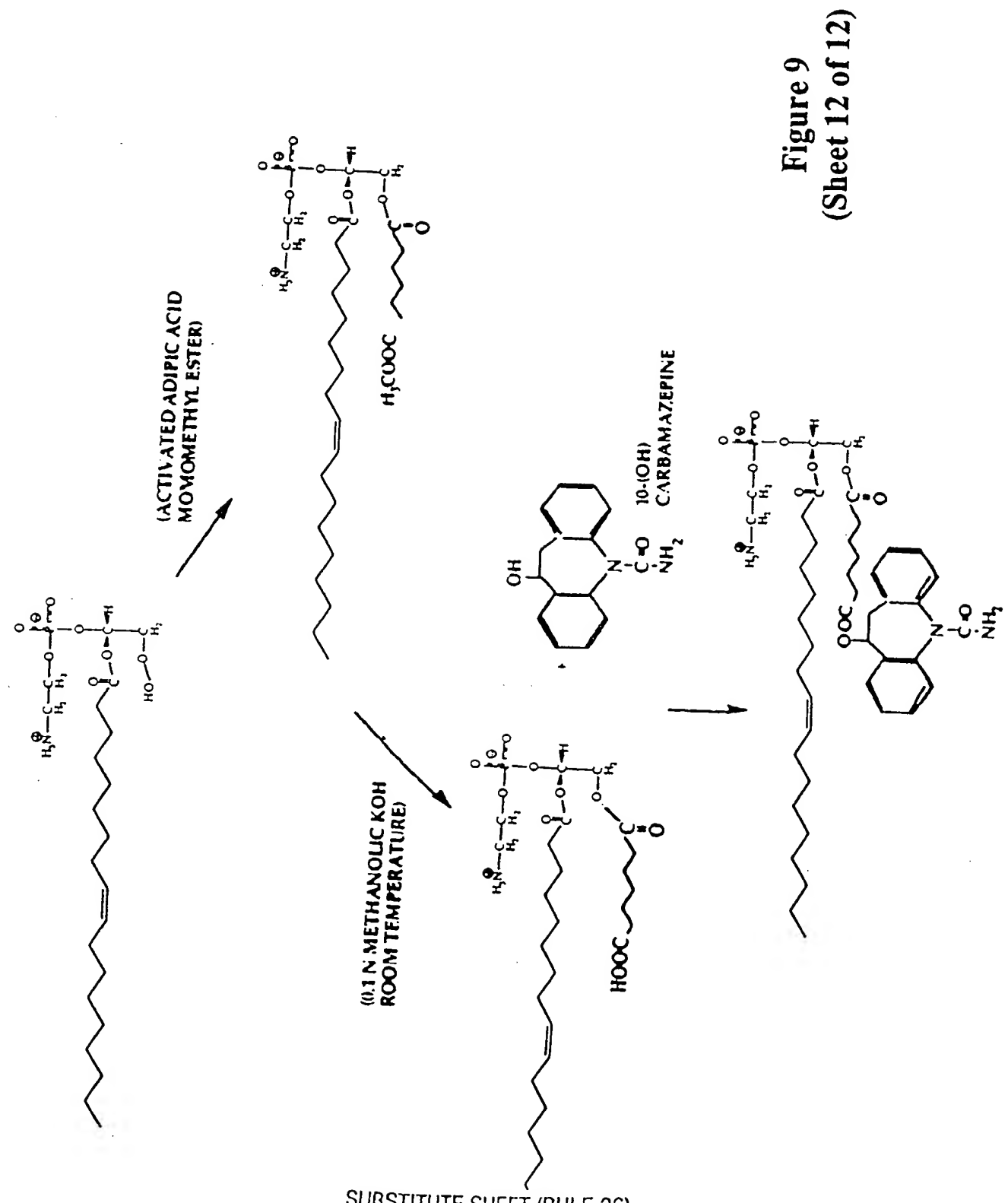


Figure 9
(Sheet 12 of 12)

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(71) Applicant: OREGON HEALTH SCIENCES UNIVERSITY [US/US]; 3181 S.W. Sam Jackson Park Road, Portland, OR 97201 (US).

(71)(72) Applicants and Inventors: YATVIN, Milton, B. [US/US]; 5226 S.W. Northwood Avenue, Portland, OR 97201 (US). STOWELL, Michael, H., B. [/US]; Catalina 438 #105, Pasadena, CA 91106 (US). MEREDITH, Michael, J. [US/US]; 3 S.W. Offenbach Place, Lake Oswego, OR 97035 (US).

(74) Agent: NOONAN, Kevin, E.; McDonnell Boehnen Hulbert & Berghoff, 300 South Wacker Drive, Chicago, IL 60606 (US).

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(57) Abstract

This invention herein describes a method of facilitating the entry of drugs into cells and tissues at physiologically protected sites at pharmicokinetically useful levels and also a method of targeting drugs to specific organelles within the cell. This polar lipid/drug conjugate targeting invention embodies an advance over other drug targeting methods known in the prior art, because the invention provides drug concentrations in such physiologically protected sites that can reach therapeutically-effective levels after administration of systemic levels much lower than are currently administered to achieve a therapeutic dose. This technology is appropriate for use with psychotropic, neurotropic and neurological drugs, agents and compounds, for rapid and efficient introduction of such agents across the blood-brain barrier. Further, the invention provides means for retention and prolonged enzymatic release of psychotropic, neurotropic and neurological drugs, agents and compounds comprising the conjugates of the invention, in the brain and central nervous system.

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INTERNATIONAL SEARCH REPORT

Inten nal Application No
PCT/US 97/19486

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K47/48

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 96 04001 A (MOLECULAR STRUCTURAL BIOTECHNO) 15 February 1996 see abstract see page 9 see page 10, line 13-15 see page 12, line 1-5; table 1 see page 21; claims 3,8,30-33	1,3-10, 12-18, 20-25
X	WO 94 22483 A (KOSM GERALD EMMANUEL ;PHARM LTD D (IL); KOZAK ALEXANDER (IL)) 13 October 1994 see abstract see page 39 - page 40; claims 1-3	1-22
Y	DE 37 26 945 A (DIETL HANS) 23 February 1989 see abstract; claim 2; examples 2,5	1-25

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[X] Further documents are listed in the continuation of box C.

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Date of the actual completion of the international search	Date of mailing of the international search report
1 April 1998	24.04.98

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	Gonzalez Ramon, N

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INTERNATIONAL SEARCH REPORT

Inter nal Application No
PCT/US 97/19486

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	WO 94 01138 A (OREGON STATE) 20 January 1994 see page 2, line 1-10 see page 8 - page 9 see page 14, line 1-10; claims 1,9-13,16,41-46	1-25
X	BIOLOGICAL ABSTRACTS, vol. 47, no. 5, 1995 Philadelphia, PA, US; abstract no. 077435, YATVIN M. B. ET AL: "Targeting lipophilic prodrugs to brain, lung and spleen" XP002061069 see abstract & KEYSTONE SYMPOSIUM ON DRUG DELIVERY: BARRIERS TO DRUG TRANSPORT AND THE DESIGN OF NOVEL THERAPEUTIC AGENTS, 7 January 1995, SOUTH CAROLINA, USA,	1,3-10, 12-18, 20-25
A	US 4 780 455 A (LIEBERMAN SEYMOUR ET AL) 25 October 1988 see abstract; claims 1,2	1-25
A	US 4 810 697 A (SPEISER PETER ET AL) 7 March 1989 see column 2, line 50 - column 3, line 68; claim 1	1-25

INTERNATIONAL SEARCH REPORT

Ir. .ational application No.
PCT/US 97/19486

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3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

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[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

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In view of the large number of compounds, which are defined by the general definition in the independent claims, the search had to be restricted for economic reasons. The search was limited to the compounds for which pharmacological data was given and/or the compounds mentioned in the claims, and to the general idea underlying the application (see Guidelines, Part B, Chapter III, paragraph 3.6).

Remark : Although claims 1-25
are directed to a method of treatment of
the human/animal body , the search has been carried out and based on the alleged effects of the compound/composition.

INTERNATIONAL SEARCH REPORT

information on patent family members

Inter nal Application No
PCT/US 97/19486

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 9604001 A	15-02-96	US 5716614 A	10-02-98
		AU 3275595 A	04-03-96
WO 9422483 A	13-10-94	AU 682029 B	18-09-97
		AU 6380894 A	24-10-94
		CA 2159610 A	01-10-94
		EP 0691852 A	17-01-96
		HU 73669 A	30-09-96
		JP 8508295 T	03-09-96
DE 3726945 A	23-02-89	NONE	
WO 9401138 A	20-01-94	US 5256641 A	26-10-93
		AU 4670093 A	31-01-94
		EP 0650371 A	03-05-95
		JP 7509227 T	12-10-95
		US 5543389 A	06-08-96
		US 5543390 A	06-08-96
		US 5543391 A	06-08-96
US 4780455 A	25-10-88	US 5002936 A	26-03-91
US 4810697 A	07-03-89	EP 0077529 A	27-04-83

WOLF, GREENFIELD & SACKS, P.C.
Attn. GATES, E.
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Boston, Massachusetts 02210
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